THE INFLUENCE OF SOME AMINO ACIDS ON NEURO-MUSCULAR TRANSMISSION

By

M. SIRSI

From the Pharmacology Laboratory, Indian Institute of Science, Bangalore

(Received March 20, 1961)

The region of neuro-muscular junction is of great importance not only in relation to muscle but also because of its many activities which are very similar to those at the synapses between different nerve neurons both in the central and in the peripheral nervous system. The phrenic nerve diaphragm preparation has assumed some importance in the study of this neuro-muscular transmission.

Normally, skeletal muscles contract when receiving impulses from the nerve centres along the motor nerve fibres and the neuro-muscular junction. Acetylcholine is considered to be the chemical mediator involved in these functions. The neuro-muscular junction appears to be highly susceptible to the influence of chemicals of divergent nature and other biological substances present in the body. While plausible explanations are available for the mode of action of certain drugs, with others, these remain still unsolved. Choline and acetylcholine in large doses block the transmission (Rosenbleuth and Simeone, 1938). Curariform drugs desensitize the end plate to acetylcholine (Robson and Keele, 1950); procaine has a similar curariform effect (Tobias, 1959); magnesium ions induce neuro-muscular block and adrenaline can temporarily relieve this block (Dybing, 1957). Adrenaline also modifies the effect of acetylcholine at the neuro-muscular junction (Burn, 1945) . Prostigmine, eserine and adrenaline potentiate the contractions produced by nerve stimulation of isolated organs. Some antibiotics, protein in nature also exert an inhibitory effect on the transmission of nerve impulses at the junction (Sirsi, 1960 unpublished observations).

Recent studies have revealed that some amino acids and their metabolic products exert considerable influence on conduction and transmission of nerve impulses in the brain and the spinal cord. The metabolism of glutamic acid and glutamine in particular have been subjected to intensive investigation. Glutamic acid has been shown to be of special significance for the functions of nervous tissue (Weil-Malharbe, 1950). It now appears that

a product of glutamic acid metabolism, aminobutyric acid may play an important part in the control of neuro-physiological activity. Aminobutyric acid blocks depolarising excitarory electrogenesis in mammalian brain, as it also blocks that of mechano-sensitive receptors and is effective in preventing certain types of seizures in mice (Purpura *et al.*, (1957a); Curtis and Phyllis, 1958) Sodium glutamate injected to ventricles on the other hand produces generalized seizures (Hayashi, 1958).

Besides glutamic acid other *w*-amino acids also exert inhibitory or excitatory effects on electrical activity of dog motor cortex. Aliphatic acids with 4 corbon atoms or less were found to be inhibitory and those with 5 or more excitatory. Glycine, has synaptic action like the longer chain amino acids (Purpura *et al.*, 1957 b).

Strong effects are thus seen to be exerted by such common amino acids as are normally present in the system. The possibility of amino acids exerting direct physiological and pharmacological effects, besides their role in protein synthesis, is now attracting considerable attention. The trophic, neurological and psychological changes that are frequently noted in states of abnormal amino acid metabolism may have a relationship to the synaptic effects of these amino acids. Synaptic as well as metabolic actions should be considered when the role of these substances in the animal is involved.

Most of the investigations carried out so far have been on the influence of the amino acids on synaptic transmissions in the brain and very little attention seems to have been given to their role in the peripheral sector. Since, the transmission at the neuro-muscular junction is considered likely to be similar in nature to the central synaptic reactions, the effects of amino acids on the neuro-muscular junction has now been studied using the rat phrenic nerve diaphragm preparation.

METHODS

The amino acids studied were leucine, nor-leucine, and *d*-alanine, glycine, *l*-phenylalanine, arginine, histidine, methionine, aspartic acid and glutamic acid.

The amino acids were dissolved in water and added to the isolated organ bath to give the required concentrations.

The test preparation used was the rat phrenic nerve diaphragm (Bülbring, 1946). This was suspended in bath of 75 ml capacity and bathed in Tyrode solution of the following composition :—

Sodium chloride,	8.0 g.
Potassium chloride,	0.2 g.
Calcium chloride,	0.2 g.
Magnesium chloride,	0.01 g.
Sodium dihydrogen phosphate,	0.05 g.
Dextrose,	2.0 g.
Sodium bicarbonate,	1.0 g.
Distilled water,	to 1 litre

The bath was kept at 35°C$\pm$1° and continuously oxygenated.

Stimulations were given by single electric shocks (rectangular in shape) every seven secs. Indirect stimulation was given through the phrenic nerve and direct contractions obtained by embedding both the electrodes in the muscle. The isotonic twiches of the muscle were recorded on smoked paper.

In the initial study, after normal contractions were recorded, different concentrations of the amino acids were allowed to remain in contact with the diaphragm for 5 mins. prior to the stimulation of the phrenic nerve. Those amino acids exhibiting alterations in the contracture were subjected to further investigation. These included the effect of the amino acids on direct stimulation, responses to stimulation immediately and on prolonged exposure of the preparation to the amino acids. The influence on the curare induced neuro-muscular block was investigated. This was obtained by adding *d*-tubocurarine choloride so as to give 1 μg/ml concentration in the bath and continuing the stimulations. When the contractions were reduced by about 50 per cent, the amino acids were added and the reversal effect on the neuro-muscular block studied.

Glutamic acid in particular, which showed a potentiating effect was examined in detail. Since glutamic acid can replace glucose in certain metabolic and functional disturbances of nervous tissue, the reactions of the test preparation to glucose was also studied.

RESULTS

Leucine, nor-leucine, arginine, histidine, *d*-alanine, *l*-alanine (Fig. 1) in doses upto 100 μg/ml did not interfere with the neuro-muscular transmission as could be deduced by the absence of any effect on the diaphragmatic contraction.

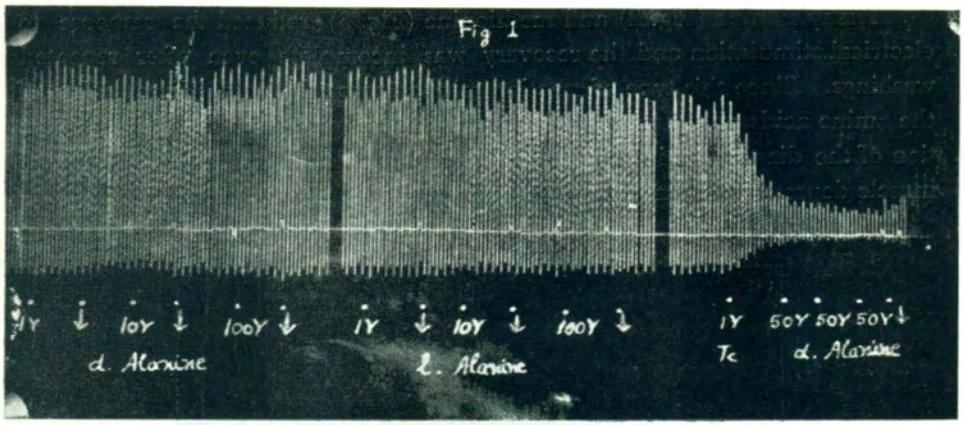

Fig. 1. Effect of *l*-alanine on rat phrenic nerve diaphragm preparation. Stimulation through nerve, every 7 sec; bath 75 ml. At arrow the bath was washed Tc, *d*-tubocurarine chloride added.

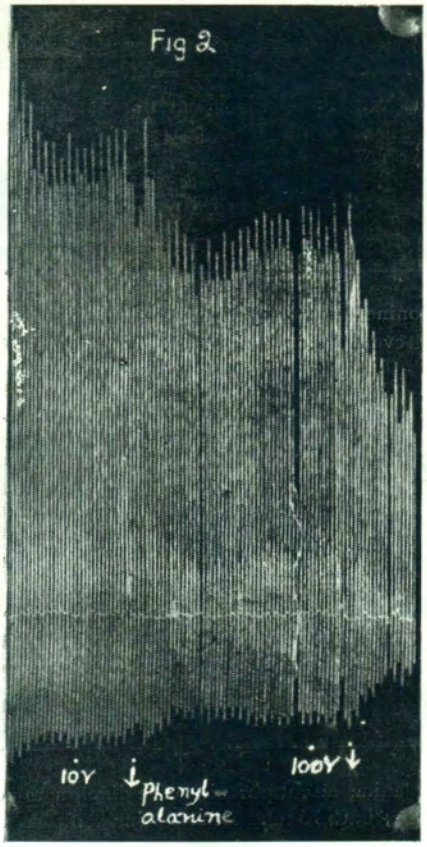

Fig. 2. Effect of phenylalanine on rat phrenic nerve diaphragm preparation. Stimulation through nerve, 1 every 7 sec; bath 75 ml. At arrow the bath was washed.

Phenylalanine (Fig. 2) and methionine (Fig. 3) reduced the response to electrical stimulation and the recovery was incomplete even after repeated washings. Since this type of reaction might also be due to a direct effect of the amino acids on the musculature, experiments were done by direct stimulation of the diaphragm in presence of the amino acids (Figs. 4a, b). No direct muscle depressant effect was seen indicating that these two amino acids pro-

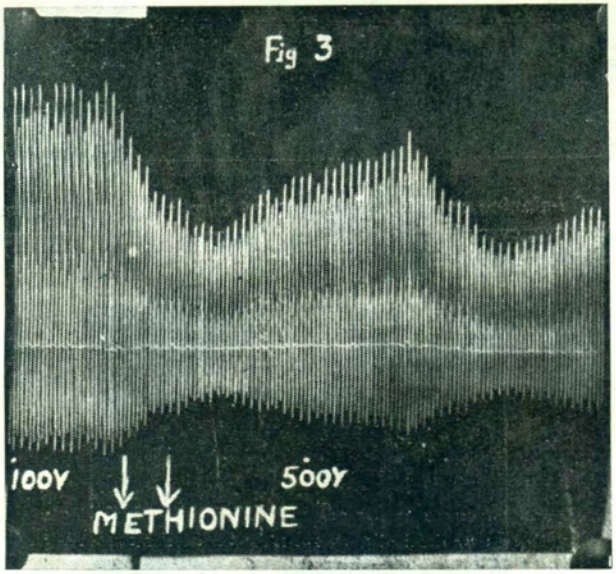

Fig. 3. Effect of methionine on rat phranic nerve diaphragm preparation. Stimulation through nerve, 1 every 7 sec ; bath 75 ml. At arrow the bath was washed.

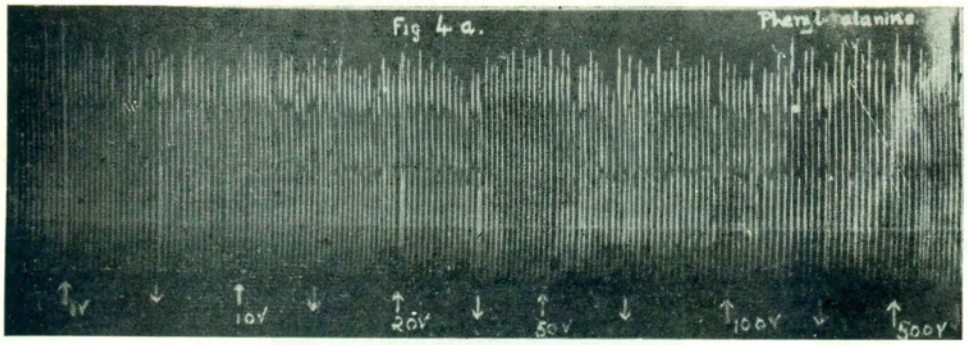

Fig. 4a. Effect of phenylalanine on rat phrenic nerve diaphragm preparation. Direct stimulation, 1 every 7 sec ; bath 75 ml. At arrow the bath was washed.

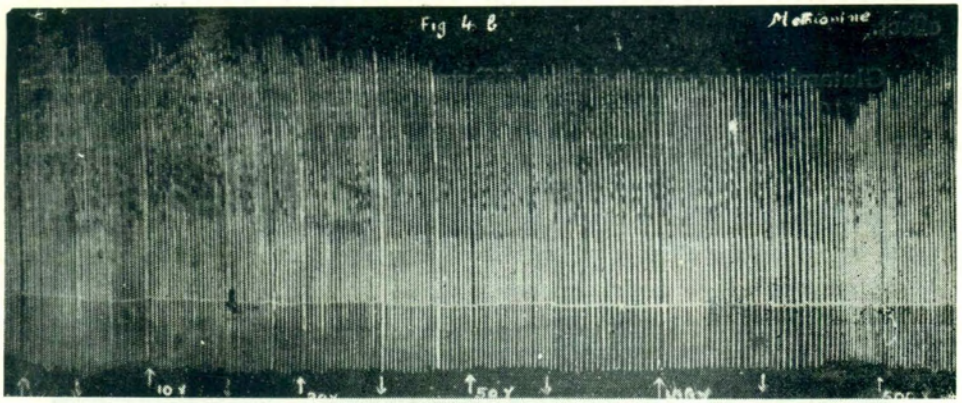

Fig. 4b. Effect of methionine on rat phrenic nerve diaphragm preparation. Direct stimulation, 1 every 7 sec ; bath 75 ml. At arrow the bath was washed.

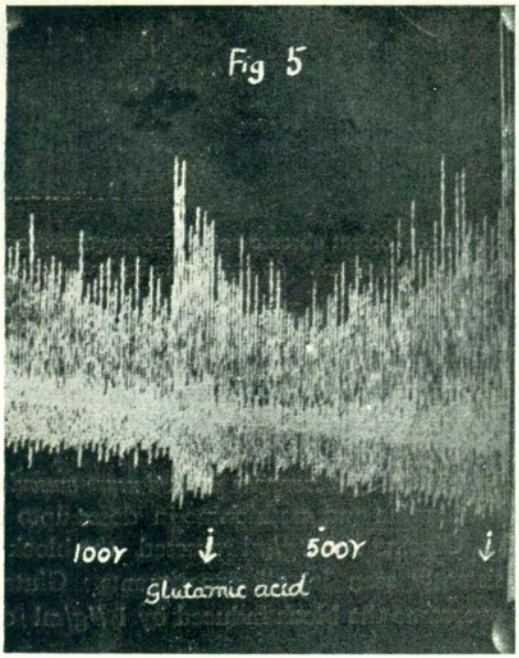

Fig. 5. Effect of glutamic acid on rat phrenic nerve diaphragm preparation. Stimulation through nerve, I every 7 sec ; bath 75 ml. At arrow the bath was washed.

bably possess a depressant effect on the neuromuscular junction which persists even after washing out the amino acids. Glycine in a concentration of 200 μg/ml reduced the response to electrical stimulation and the recovery was in

complete even after repeated washings. Aspartic acid with 100 μg/ml had no effect.

Glutamic acid exhibits quite a different pattern of action. In concentrations of 100 μg/ml and above, the amino acid potentiated the nerve stimulation (Fig. 5). This effect was not due to altered sensitivity of the musculature since no potentiation was seen on direct stimulation (Fig. 6): it was also observed that the effect was not the result of an altered pH of the perfusion fluid, by noting the absence of any stimulatory effect when a phosphate buffer of the same pH as glutamic acid was added to the bath.

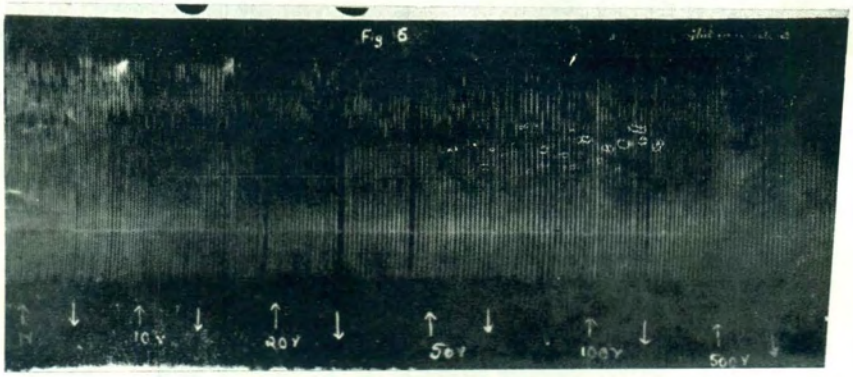

Fig. 6. Effect of glutamic acid on rat phrenic nerve diaphragm preparation. Direct stimulation, 1 every 7 sec ; bath 75 ml. At arrow the bath was washed.

On further detailed study, it was seen that the potentiating action was more prominent when glutamic acid was allowed to remain in contact with the nerve preparation for 5 minutes and above and not apparent when stimulations were given immediately on addition (Figs. 7,8)

Influence of amino acids on d-tubocurarine induced neuro-muscular block.—Leucine, norleucine (Fig, 9) *d*-alanine and methionine in doses upto 100 μg/ml had no effect on the block. Glycine 100 μg/ml reversed the block (Fig. 10) but the effect was not consistently seen in all experiments. Glutamic acid in 100 μg/ml and above overcame the block induced by 1 μg/ml of *d*-tubocurarine. (Fig. 10).

Since glutamic acid is shown to replace glucose in some of its metabolic activities (Tsctrirgi *et al.*, 1949), the effect of glucose on neuro-muscular junction was studied (Fig. 11). In much lower concentrations than glutamic acid, glucose potentiated the indirect stimulation. This could be observed even at 1 μg/ml of the perfusion fluid.

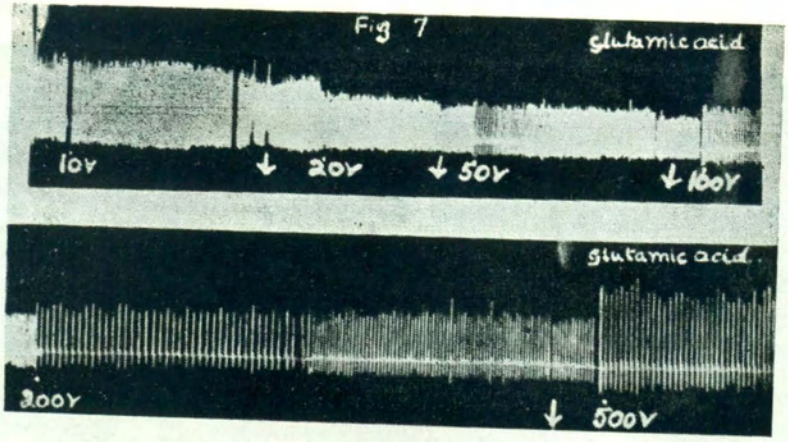

Fig. 7

Fig. 7,8. Effect of glutamic acid on rat phrenic nerve diaphragm preparation. Stimulation through nerve, 1 every 7 sec; Fig. 7, stimulated after glutamic acid was kept in contact with the muscle for 5 min, and in Fig. 8 immediately on addition of the amino acid. At arrow the bath was washed.

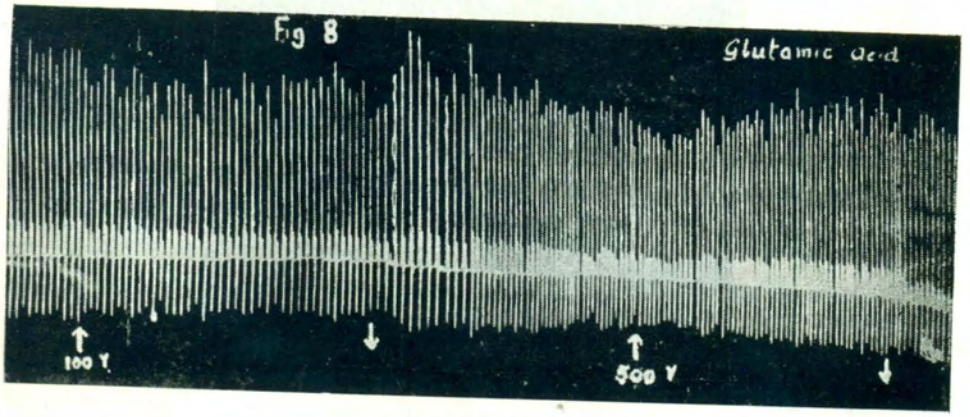

Fig. 8

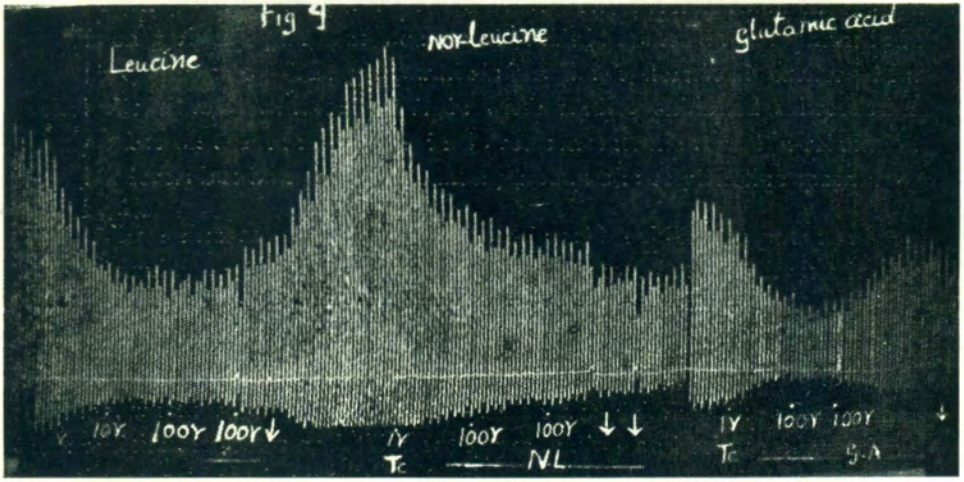

Fig. 9. Effects of leucine, nor-leucine and glutamic acid on rat phrenic nerve diaphragm preparation after *d*-tubocurarine induced neuro-muscular block. Stimulation through nerve, 1 every 7 sec; bath 75 ml. At arrow the bath was washed. Tc, *d*-tubocurine chloride added.

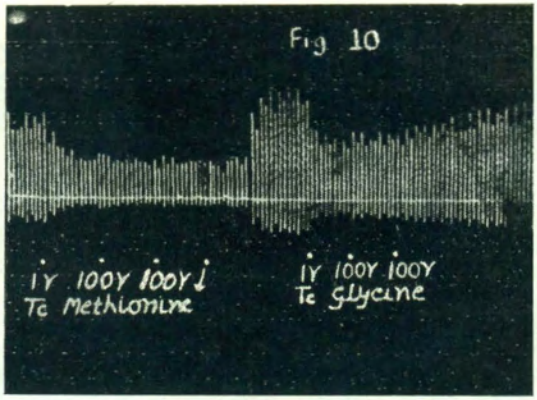

Fig. 10. Effects of methionine and glycine on rat phrenic nerve diaphragm preparation after *d*-tubocurarine induced neuro-muscular block. Stimulation through nerve, 1 every 7 sec; bath 75 ml. At arrow the bath was washed. Tc, *d*-tubocurarine chloride added.

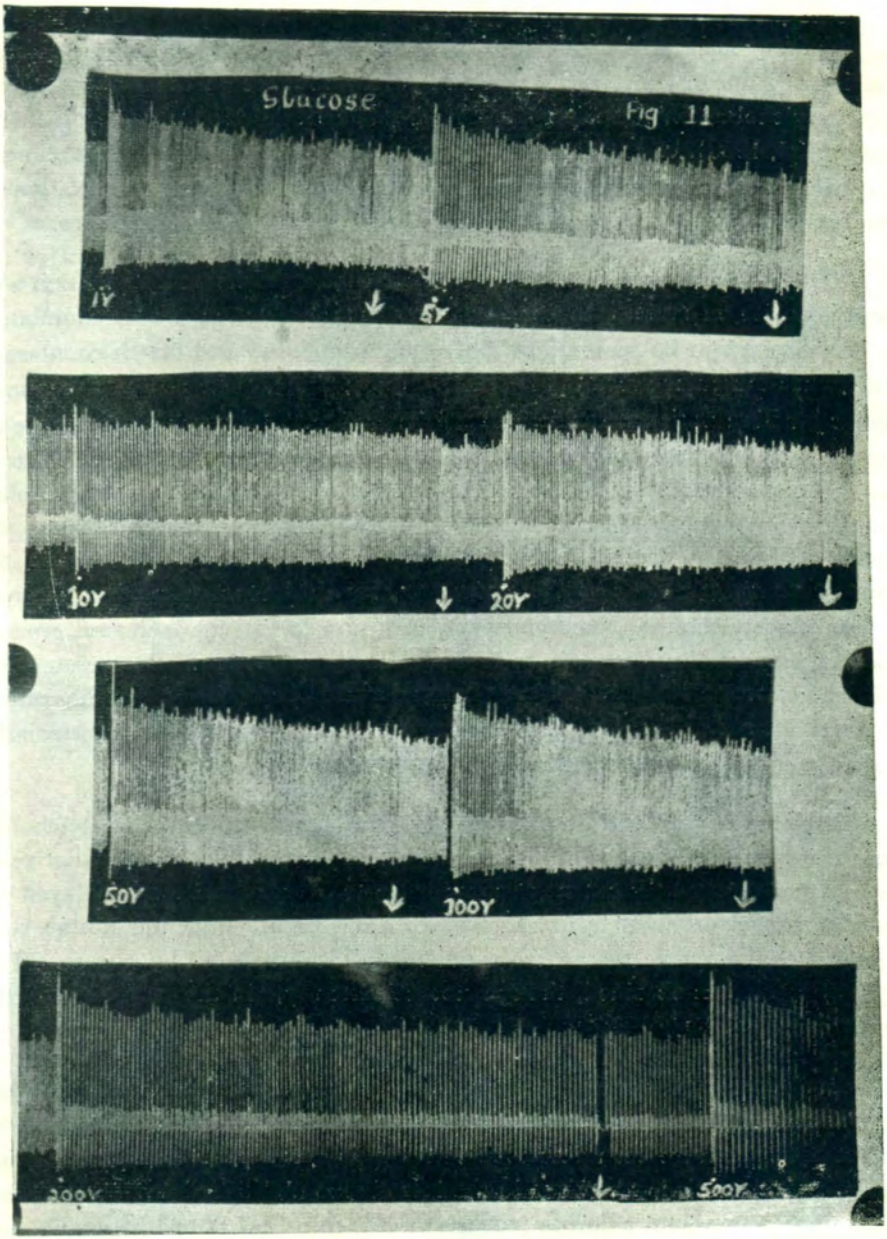

Fig. 11. Effect of glucose on rat phrenic nerve diaphragm preparation. Stimulation through nerve, 1 every 7 sec; bath 75 ml. At arrow the bath was washed.

DISCUSSION

Effect of amino acids on smooth musculature. The effect of some of these amino acids on guineapig and rat ileum and their influence on the spasms induced by acetylcholine on these preparations has been described earlier (Murthy and Sirsi, 1959). Glutamic acid, in these studies exhibited a direct stimulatory effect on the musculature in lower concentrations and a relaxation at higher levels but had no potentiating action on acetylcholine spasms.

These observations, viz., that glutamic acid exhibits no potentiation with acetylcholine on smooth muscles and also has no effect on direct stimulation of the diaphragm but potentiates the nerve stimulation and that it counteracts the neuro-muscular block induced by *d*-tubocurarine, points—the neuro-muscular junction as the probable site of its action. As regards the mode of action, whether the amino acid exhibits an acetylcholine like effect, or acts as an anticholinesterase or facilitates the production and release of acetylcholine at the neural junction needs elucidation. Since the potentiating effect of glutamic acid is better seen when it is left in contact with the preparation for some time and glutamic acid is known to play an important part in the synthesis of acetylcholine (Nachmansohm and John, 1945), the increased production of acetyloholine seems to be its likely mode of action. Eccles interprets the phenomenon of potentiation as being derived from changes in the rate of process whereby the presynaptic unit makes and stores the transmitter substance at the synapse and makes it available at the synapse.

While glutamic acid potentiated the stimulatory effect of acetylcholine and neutralised the action of *d*-tubocurarine, glycine exhibited a peculiar reaction. It depressed the extent of contraction to nerve stimulation, delayed the rate of recovery even after its removal from the bath. But, the amino acid could partially overcome the neuro-muscular block induced by curare. This type of result was obtained, though not consistently, in the majority of the experiments.

Such compounds which are inert in potentiating the action of acetylcholine but yet posessing the typical cholinergic property of antagonizing the effect of curare have been described earlier (Cowan, 1938). The investigation of such compounds has resulted in the introduction of 3-hydroxyphenyl dimethyl-ethyl ammonium chloride (edrophonium chloride) as a diagnostic and therapeutic drug for myasthenia gravis (Westberg *et al*, 1951). Whether a similar role could be attributed to glycine requires further investigation.

These preliminary studies indicate that the neuro-muscular junction can be influenced by some amino acids. The increased excitability or the diminished contractile power of the musculature seen in disturbed metabolic functions may possibly be attributed to the amino acid alterations in the system. The potentiating effect of glutamic acid, more marked on prolonged contact, at the neuro-muscular junction and its ability to reverse the curariform block are observations which emphasise that glutamic acid plays a prominent role not only in the central nervous system but also in its peripheral ramifications. This is further confirmed by the observation that the spinal reflex due to the absence of glucose in the perfusion fluid may be regained by incorporating glutamic acid (Cowan 1938) ; probably the neuro-muscular junction and the central synaptic regions respond in a similar manner to these environmental alterations.

SUMMARY

The effects of several amino acids on the neuro-muscular junction were studied, using rat phrenic nerve diaphragm preparation.

A significant observation has been, the influence of glutamic acid which potentiated the action of nerve stimulation, more marked on prolong contact, and its ability to reverse the curariform block. It is suggested that glutamic acid plays a prominent role on neuro-muscular junction.

The author wishes to express his thanks to Mr. Shankara Sastry, Mrs. Nagaratna and Mr. Gopinath for technical assistance.

REFERENCES

Bülbring, E. (1946). *Brit. J. Pharmacol.*, **1**, 38.
Burn, J.H. (1945). *Physiol. Rev.*, **25**, 377.
Cowan, S.L. (1938). *J. Physiol.*, **93**, 215.
Curtis, D.R. and Phyllis, J.N. (1958). *Nature*, **182**, 322.
Dybing, F. (1957). *Acta. Pharmacol. Toxicol.*, **13**, 80.
Hayashi, T. (1958). *Nature*, **182**, 1076.
Nachmansohm, D. and John, H.M. (1945). *J. Biol. Chem.*, **158**, 157.
Purpura, D. P., Martin, G. and Grundfast, H. (1957 a). *Science*, **125**, 1200.
Purpura, D. P., Martin, G. and Grundfast, H. (1957 b). *P.S.E.B. & M.* **95**, 791.
Rosenbleuth, A. and Simeone, M. (1938). *Am. J. Physiol.*, **122**, 708.

Robson, J.M. and Keele, C.A. (1950). *Recent advances in Pharmacology* London, p. 23, J.A. Churchill.

Suryanarayana Murthy, P. and Sirsi, M. (1959). *Ind. Med. Rec.*, **79**, 77.

Tobias, J.M. (1959). *Ann. Rev. Physiol.*, **21**, 299.

Tsctrirgi, R.D., Gerard, R.W., Jenerick, H., Boyarsky, L.L. and Hearor, J.Z. (1949). *Fed. Proc.*, **8**, 166.

Weil-Malharbe, H. (1950). *Physiol. Rev.*, **30**, 549.

Westberg, M.R., Majee, K.R., and Shideman, F. E. (1951). *Univ. Mich. Med. Bull.*, **17**, 311.